PRIORITY RESEARCH NEEDS
Toxicology and Metabolic Consequences Working Group

Introduction

The Toxicology and Metabolic Consequences Working Group was one of five working groups convened during the JIFSAN/NCFST-organized Workshop on Acrylamide in Food, held in Chicago on October 28-30, 2002. TheWorking Group reached conclusions regarding data gaps and consequent research needs in each of six focus areas: metabolism and kinetics, genetic toxicity, reproductive/developmental toxicity, carcinogenicity, neurotoxicity, and epidemiology. Data gaps were characterized as areas where additional data (new, improved, etc.) would be most likely to significantly improve our ability to evaluate the potential adverse health effects from exposure to acrylamide in food.

Data gaps/research needs in each of the six focus areas were culled and prioritized to identify the nine most critical research needs, highlighted under the focus areas below. It was the opinion of the Working Group that these top-priority research initiatives would not require extensive method development and that most could be accomplished in a relatively short time (6-12 months from commencement).

One theme of the prioritized research is *to assess the significance of adverse effects observed at high doses for low-level human exposure in foods*. For example, neurotoxicity studies at dose levels orders of magnitude higher than likely human exposures in foods have suggested the possibility that cumulative neural damage may result from long-term exposures at lower levels, so chronic rodent studies designed to investigate this possibility were given a higher priority. In order to link the results of rodent studies to human risks, kinetics and metabolism studies in humans and the identification of appropriate dose metrics for the major toxicity endpoints were identified as critical research needs.

Another theme is *the assessment of the significance for humans of effects observed in vitro or in vivo in rodents.* For example, in rats thyroid follicular cell tumors were increased in both acrylamide carcinogenicity studies. For some chemicals these tumors have been shown to be produced by a mechanism that does not occur in humans and the tumors have therefore been considered irrelevant in assessing human cancer risk. This possibility needs to be investigated for acrylamide. Also, germ cell mutations have been observed experimentally *in vitro* and *in vivo* but have not been reported in humans, so a prioritized research need is to search for these effects in the sperm of highly exposed workers in known cohorts.

Metabolism and Kinetics

Information on the metabolic fate of acrylamide is important to informing extrapolation of health effects from animal data to human health risk. These data are also central to predicting toxic responses at low doses on the basis of those observed at high doses. In this regard data on the metabolic fate of acrylamide from animal test species, particularly the rat, are fairly well established. However, there is a need for **further information on the critical events and dose metrics related to the mode(s) of action at relevant doses for the key toxicities of acrylamide.**

In contrast to the animal data, there exists relatively little, if any, data on metabolic fate and kinetics of acrylamide in humans. Therefore the work group recommended as a top priority **the collection of metabolic fate and kinetic data in humans, including bioavailability from foods.** Methodologies using high sensitivity and resolution bioanalytical instrumentation, coupled with stable isotope techniques should facilitate the collection of these data. It is anticipated that these results could be collected within 6-12 months from the time the project begins.

Other specific elements of research programs aimed at improving understanding of metabolism and kinetics in humans include:

- Assessing the kinetic determinants of potentially susceptible subpopulation
- Improve existing physiologically-based pharmacokinetic models (e.g. quantify rate constants for use in kinetic modeling)
- Assessing kinetic determinants across developmental stages
- Molecular and kinetic characterization of binding to sulfhydryls in target and non-target sites (e.g. measure rate constants for binding to critical targets vs. glutathione)
- Compare and contrast endpoints of acrylamide toxicity to other other –SH active agents

Genetic Toxicity

Data from traditional genetic toxicity studies are well described for acrylamide. The data suggest several possible mechanisms by which genetic damage could affect two of the more important adverse health effects suspected from acrylamide exposure (i.e. cancer and heritable genetic damage). Further elucidation of which of the several possible mechanisms for inducing genetic damage is operating will guide approaches to assessing health risks to humans from dietary exposure to acrylamide. In this regard the working group supported **investigations of the formation of adducts of acrylamide or glycidamide with DNA and significant nuclear proteins (for example, protamine or chromosomal motor proteins), especially at critical target sites such as sites of tumor formation and male germ cells.** Research regarding classification, biological relevance, and the kinetics and activity of adducts, if they occur, is needed to inform potential mode of action development. Species differences, *in vivo* vs. *in vitro* formation, and formation at relevant dose ranges for such adducts are particularly important areas for research. Improvements in current bioanalytical methods for detecting DNA and protein adducts may be necessary to address this line of research at dose levels relevant to those used in animal bioassays and those found in foods.

Other important research needs in this area include:

- Use of specific genetically modified mouse strains (e.g. thymidine kinase knockout and Big Blue (*LacI/lacZ*) mouse models) to assess the mode of genotoxic damage *in vivo*.

Reproductive and Developmental Toxicity

Significant decreases in the number of live pups were observed when acrylamide was administered to parental rats and mice. Dominant lethality studies show this finding

of decreased litter size to be likely related to germ cell toxicity. While a NOAEL for pre- and post-implantation loss can be determined from the multigenerational studies, it is expected that effects on the predominant precursor event, germ cell toxicity, would occur at lower doses. At present, germ cell toxicity studies are single, high dose studies. Therefore, to assess low dose reproductive toxicity and to establish a NOAEL for germ cell effects, a priority research need is **to develop dose-response data for germ cell toxicity that includes consideration of the relevant doses for acrylamide ingestion in food.**

Acrylamide is a neurotoxicant in several species, including man, and signs of neurotoxicity (e.g., grip strength) have been noted in offspring of mice treated with acrylamide. The potential for developmental neurotoxicity in offspring following oral acrylamide exposure of dams during gestation has been evaluated in two rat studies using a limited battery of gross behavioral tests (open field activity, auditory startle habituation, passive avoidance), which did not show effects in offspring. However, other tests and more sensitive indicators of developmental toxicity are needed **to improve the weight-of-evidence regarding neurodevelopmental effects at doses relevant to those expected in food and to establish a NOAEL for those effects.**

Other important research needs in this area include:

- Dominant lethal study in CYP2E1 knockout mice to assess the role of glycidamide in germ cell toxicity and to further refine relevant dose-response relationships
- Developmental toxicity study in a non-rodent species, which should include acquiring toxicokinetic data.

Carcinogenicity

The weight of evidence for carcinogenic potential of acrylamide in humans is based primarily on animal data with limited evaluation in human populations. Although several studies of acrylamide carcinogenicity exist, several questions remain that pertain to the interpretation of observations of tumors from these studies. The working group felt that additional research activities could improve the utility of these studies and reduce uncertainties related to carcinogenic potential. The first activity would be **to conduct a pathology working group meeting in which the histology slides from the animal bioassays would be reviewed by pathology experts using updated diagnostic criteria.** The objective would be to develop a consensus view on diagnoses related to key neoplastic lesions.

The second activity would be **to investigate the mechanism of thyroid tumor induction by acrylamide.** Thyroid tumors were among the most prominent of tumor types diagnosed in the rat cancer bioassays. However, more recent research and guidelines from IARC and the USEPA suggest that certain mechanisms by which thyroid tumors are induced in rats are of questionable relevance to humans. The objective of this research would be to determine the operative mechanism for acrylamide and, thereby, clarify the relevance of these tumors to humans. It is expected that this research could be completed within 6 months.

Other important research needs in this area include, if additional long-term bioassays are considered:

- Evaluation of carcinogenicity under conditions including perinatal exposure

- Evaluation of the role of glycidamide using the CYP2E1 knockout mouse in mechanistic studies supplementing the long-term bioassay, for example by examination of hormone effects or adduct formation in subchronic experiments
- Assessment of other mechanistic issues and inclusion of neurotoxicity evaluations in the long-term bioassay, if feasible.

Neurotoxicity

The working group discussed the various known neurotoxic effects of acrylamide at length. Notably, neurotoxicity is the only toxic response to acrylamide exposure known to occur in humans. Issues include the relevance of rodent models for studying these effects and the dose levels over which mechanisms of neurotoxicity that are relevant to humans may be expressed. Since the neurotoxic properties of acrylamide in humans are known principally from occupational studies of adults at exposure levels that are high relative to our current understanding of dietary exposures, it was felt that the possibility of a similar effect in children warranted research first in animals and possibly subsequent epidemiological follow-up. The animal research proposed was **evaluation of the relationship between dose, duration, and effect-levels and onset of neurotoxicity.** This would include light microscopic (silver staining as well as routine histopathology) and ultrastructural analysis of synaptopathy and axonopathy, linked to nerve function, behavior, and reversibility of effects. Evaluation of exposure durations longer than 3 months is particularly needed. Studies of this type could yield informative data within six months but full completion of the program would likely require at least two years from commencement.

Other important research needs in this area include:

- Evaluation of existing ultrastructure pathology results for evidence of peripheral neuropathy (e.g. Johnson et al. study)
- Assessing potential additive effects to other pre-existing neurological disease using methods such as *in vitro* neurite extension and animal models for multiple sclerosis and amyotrophic lateral sclerosis diseases.

Epidemiology

Peripheral neuropathy has been investigated in workers exposed to acrylamide and this is the one clear effect in humans of high dose exposures to acrylamide. The potential carcinogenicity of acrylamide in humans has been examined in worker cohorts with generally negative results, but the power of the studies was limited. Designing studies of cancer and acrylamide exposures in food will be very difficult, given the relatively low level of exposures and the many potential confounders that will be hard to quantify. However, the potential for reproductive toxicity in human populations has not yet been studied, and this endpoint may be easier to evaluate with respect to confounding. A defined research need is **to design and conduct new epidemiology studies to evaluate sperm chromosomal abnormalities (morphology and quality, if practical) in previously evaluated worker cohorts or other highly exposed populations.** Sperm chromosomal abnormalities would serve as a biomarker for potential reproductive toxicity, and, if found, may require further study. Understanding the relationship between hemoglobin adducts (or other markers of exposure) to chromosomal aberrations would also be useful.

Other important research needs in this area include:

- Evaluation of variation in hemoglobin adduct levels (or other markers of exposure/effect) with sister chromatid exchange (and other markers of chromosomal effects)
- Investigation of the onset and development of neurotoxicity and the potential for cumulative effects and/or long-term effects
- Evaluation of existing surveillance studies (e.g. medical monitoring data) in occupational cohorts for additional data on exposure levels that do and do not cause neurotoxicity
- As a longer-term research need, development of a prospective study using large population surveys, e.g. NHANES or EPIC, in which acrylamide exposure (possibly assessed through hemoglobin adducts) in non-occupational populations would be studied.